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Spectrophotometric Determination of Rifampicin and Isoniazid in Pharmaceutical Preparations

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Three simple spectrophotometric methods for the determination of rifampicin (RIF) and isoniazid (INH) in pharmaceutical preparations have been developed. First method is based on the determination of graphical absorbance ratio at two selected wavelengths. In the second method, derivative spectroscopy is used to eliminate spectral interference and the third method is based on additivity of absorbances. All the three methods were found to be simple, rapid, accurate and can be adopted in routine analysis of drug in formulations.

Rifampicin (RIF) and isoniazid (INH) are widely used in the treatment of tuberculosis. These drugs are official in IP[1,2], BP[3,4] and USP[5,6]. The IP suggests a microbiological assay method for RIF and a titrimetric method for INH. The other methods available in the literature are ion pair extraction[7], colorimetric[8], HPLC[9], chelate formation[10], and HPTLC[11] for RIF and spectrophotometric[12], HPLC[13] and colorimetric[14], for INH. RIF and INH in combined dosage form is official in USP. Few simultaneous methods of analysis have been reported which include HPLC[15], RPHPLC[16], LC and spectrophotometric[17]. Although several methods are available for simultaneous estimation of these two drugs, the present work deals with the development of simple, rapid, reproducible methods for simultaneous estimation RIF and INH using spectrophotometry. All the three methods described here are simple, accurate, reproducible and economical with the advantage that there is no need of prior separation of both the components.

EXPERIMENTAL

A Shimadzu UV 1601 recording spectrophotometer was used for experiments with 10 mm matched quartz cells. Stock solution (1 mg/ml) of RIF and INH were prepared in methanol. Working solutions were prepared by appropriate dilutions of the stock solution in methanol.

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Method I, graphical absorbance ratio method:

Graphical absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoaborptive point and the other being the wavelength of maximum absorption of one to the two components from the overlain spectra of two drugs (fig. 1). It is evident that RIF and INH shows an isoabsorptive point at 281 nm. At 340 nm the absorbance of RIF is maximum where INH shows zero absorbance. Six mixed standard solutions with concentration 0, 1.5, 3, 4.5, 6, 7.5 µg/ml of RIF and 7.5, 6, 4.5, 3, 1.5, 0 µg/ml of INH were prepared in methanol and the ratio of absorbances at 281 nm and 340 nm were plotted against the relative concentration to obtain calibration curve. The curve shows linearity in the concentration range of 0-7.5 µg/ml for both the drugs. Linearity data consisted of a slope of 0.284, an intercept at 0.017 and a correlation coefficient of 0.999.

Method II, derivative spectrophotometric method:

First derivative spectra of the two drugs were recorded (fig. 2) and it was observed that RIF and INH showed zero absorbance at 242.6 nm and 261.6 nm, respectively. As at the zero crossing point on the first derivative spectra of on drug, the other drug showed significant absorbance, these

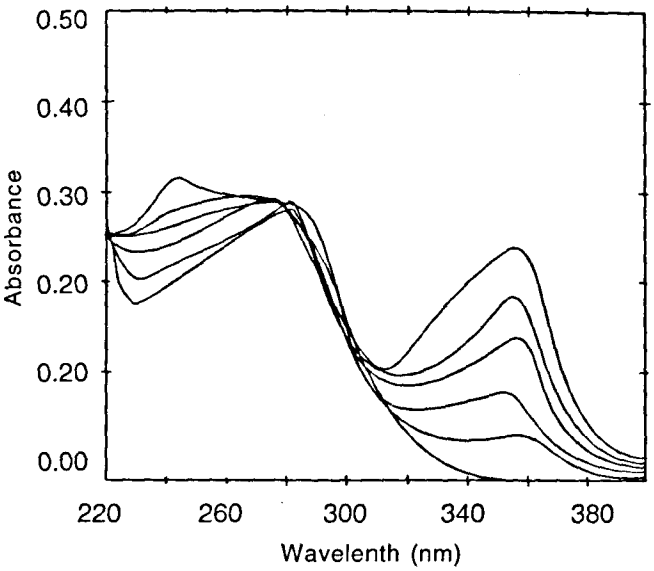

Fig. 1: Overlain spectra of mixed standards.

Overlain spectra of six mixed standards of rifampicin and isoniazid.

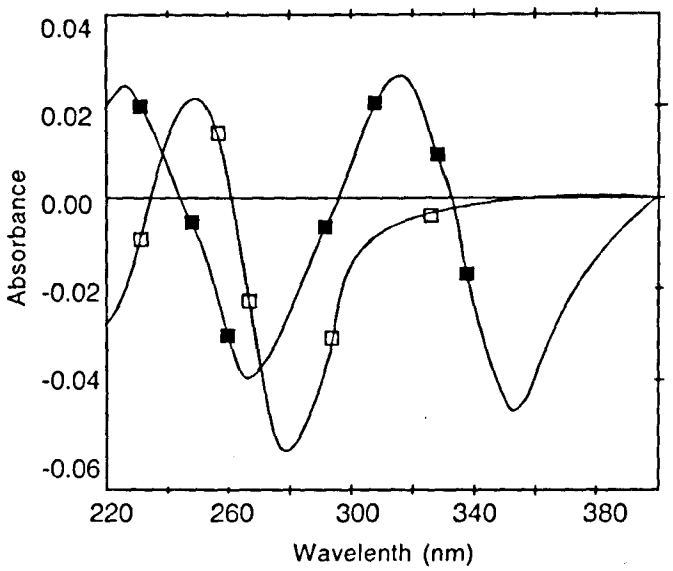

Fig. 2: First derivative overlain spectra.

First derivative overlain spectra of rifampicin (—■—) and isoniazid (—□—) and the sample size is 8.

two wavelengths were employed for the estimation of RIF and INH respectively (key entry N=8, available in Shimadzu 1601 A) without any interference. The calibration curves were plotted at these two wavelengths (242.6 and 261.6 nm) using different concentrations of RIF and INH against absorbances which showed linearity. The least square method was used for determination of slope which was RIF – 0.006, INH – 0.004, the intercept 0.006 and 0.004 and the coefficient of correlation for RIF 0.998 and INH 0.998, respectively.

Five mixed standard solutions with concentrations 0, 15, 30, 45, 60 µg/ml of RIF and 60, 45, 30, 15, 0 µg/ml of INH were prepared in methanol. All five mixed standard solutions were scanned in the wavelegth between 220 nm to 400 nm. The spectral data from these scans were used to determine the concentration of the two drugs in the tablet and capsule sample solutions. The curve shows linearity in the concentration range of 0 to 60 µg/ml for both the drugs.

Method III, based on property of additivity of absorbances:

Overlain spectra of standard solution of FIF and INH were scanned. The two wavelengths on the RIF curve was found out where it showed same absorbance. The two wavelengths were 340 nm and 266 nm. At 340 nm RIF showed maximum absorbance where INH showed zero absorbance (fig. 3). Mixed standard solutions of different concentrations were then scanned. The calibration curves were plotted against respective absorbances at 340 nm. For RIF, the absorbance for determination of INH was taken as $A^1 = A_{266} - A_{340}$ where, A^1 is the absorbance difference taken for plotting calibration curve of INH. A_{266} is the absorbance contribution by both drugs at 266 nm A_{340} is the absorbance by RIF (where INH shows zero absorbance at 340 nm).

Six mixed standard solution with concentration 4.5, 9, 13.5, 18, 22.5, 27 µg/ml of RIF and 3, 6, 9, 12, 15, 18 µg/ml of INH were prepared in methanol and scanned in the range of 220 nm to 400 nm. The calibration curves were plotted as per procedure discussed earlier. The curve shows linearity in the concentration range of 0-27 µg/ml for RIF and 0-18 µg/ml for INH.

Estimation from tablets and capsules:

Twenty tablets and capsules were weighed separately and powdered. The powder equivalent to 450 mg of RIF and 300 mg of INH was taken and dissolved in 100 ml methanol. Dilution of this solution was carried out in such away that the final concentration was 4.5 µg/ml of RIF and 3.0 µg/ml of INH, for methods I and III and 45 µg/ml of RIF and 30 µg/ml of INH for the method II. The analytical procedure was then carried out as discussed earlier. In all the three methods results were directly obtained from the calibration curve, these are given in the Table 1.

To determine the precision and accuracy of the above

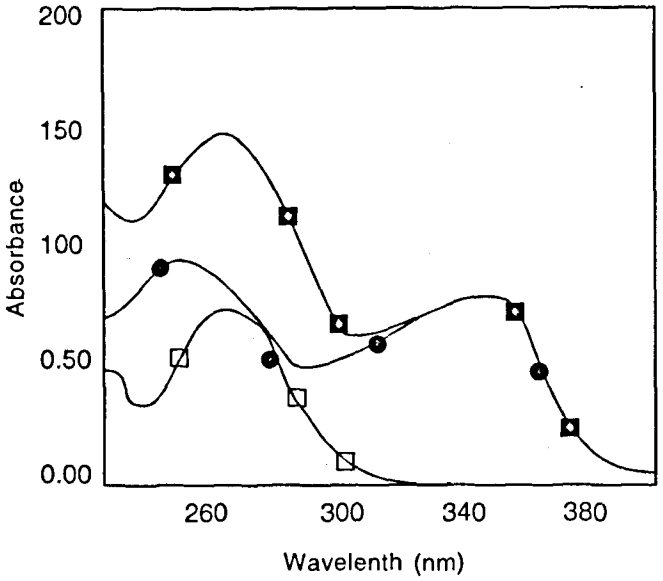

Fig. 3: Overlain spectra of individual drugs and their mixture.

Overlain spectra of rifampicin (—●—) and isoniazid (—□—) and a mixture of both (—■—).

methods, recovery experiments were performed using the method of addition. A fixed volume of standard solution was added to different concentrations of the sample solutions. The total amount of drug was then determined by these three methods and the amount of added drug found by difference. The results of recovery study are given in Table 2.

RESULTS AND DISCUSSION

All the three methods were found to be accurate, simple and rapid for routine simultaneous analysis of the formulations without prior separation. The first method employed the graphical absorbance ratio at two selected wavelengths and once the absorbance ratio is determined, the next step is determining the concentration of the drugs from the calibration curves. The second method is used to eliminate the spectral interference from one of the two drugs while estimating the other drug by selecting the zero crossing point on the derivative spectra of each drug at the selected wavelengths. In the third method two wavelengths of RIF absorbance curve that (λ_1= 340 nm, λ_2= 266 nm) have same absorbance were chosen. The content of RIF was directly found from the first wavelength (λ_1). The sec-

TABLE I: RESULTS OF COMMERCIAL SAMPLE ANALYSIS.

Sample tab/cap	*Percent found USP Method	Method I		Method II		Method III	
		Percent Estimated**	CoV	Percent Estimated**	CoV	Percent Estimated**	CoV
Tab-I	R - 99.90	101.02±0.46	0.46	99.36±1.53	1.53	99.22±0.70	0.70
	I - 100.15	99.06±0.43	0.43	99.73±2.14	2.15	99.69±1.22	1.22
Tab-II	R - 100.10	101.58±0.37	0.37	100.18±1.02	1.01	100.07±1.00	1.00
	I - 99.90	98.77±0.41	0.42	99.73±1.48	1.48	99.03±0.83	0.84
Cap-I	R - 100.50	100.64±0.48	0.48	102.28±0.78	0.76	102.47±0.69	0.67
	I - 100.3	99.19±0.48	0.48	100.45±1.80	1.79	99.54±1.13	1.14

Asterisk denotes mean of 3 determinations while double asterisk indicates mean and standard deviation of 6 observations. CoV stands for coefficient of variance and R and I represent rifampicin and isoniazid, respectively. Tab stands for tablets while cap denotes capsules. Label claim for R and I are 450 and 300 mg/tab or cap, respectively.

TABLE 2: RECOVERY STUDY OF COMMERCIAL SAMPLES BY PROPOSED METHODS.

Sample	Percent Recovery (Mean±SD*)					
tab/cap	Method I		Method II		Method III	
	RIF	INH	RIF	INH	RIF	INH
Tablet -I	98.50±0.91	99.95±0.35	99.77±0.22	99.27±0.85	99.38±0.38	98.72±0.79
Tablet -II	98.47±0.71	98.52±0.95	99.58±0.07	99.12±0.80	99.11±0.60	99.84±1.85
Capsule -I	100.30±1.65	99.09±1.04	101.13±1.25	101.31±1.18	100.50±1.79	98.64±0.60

Asterisk denotes that each value is a mean of 4 observations with standard deviation (SD). RIF stands for rifampicin and INH for isoniazid.

ond wavelength (λ_2) is used for determination of INH from which the absorbance of RIF was subtracted and a calibration curve was plotted. The development of these methods is based using a spectrophotometer with different modes. The results obtained by using these three methods when compared with the USP method and the results of the recovery study indicate that these methods are accurate and reproducible. Among the three methods, the derivative spectrophotometric method appears to be the best since it is found to be simple and more accurate compared to the other two.

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